The Development of the Parasite of Oriental Sore in Cultures.

By

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With Plate 20.

[The following memoir is made up from two communications, dated respectively December, 1908, and January, 1909, which I received from Dr. Row. Each letter was accompanied by sketches and stained preparations; from the latter the figures on Pl. 20 have been drawn by my assistant, Miss Rhodes, at the Lister Institute. The account given here is, for the most part, in Dr. Row's own words; any remarks of mine are in square brackets.

The parasites causing oriental sore were first described accurately by Wright ('Journ. Med. Research,' vol. x, 1903, p. 472), and named by him Helcosoma tropicum. On account of their obvious affinity (which the present memoir confirms) with the Leishman-Donovan bodies of kala-azar, Wright's bodies have been referred by subsequent writers (compare Lühe in Mense's 'Handbuch der Tropenkrankheiten,' iii, 2, 1906, p. 203) to the previously established genus Leishmania Ross ('Brit. Med. Journ.,' 1903, vol. i, pp. 1261 and 1401), and they now stand as Leishmania tropica (Wright), the only other known species of the genus being L. donovani (Lav. et. Mesn.), the parasite of kala-azar.

It was first discovered by Rogers ('Quart. Journ. Micr. Sci.,' vol. 48, 1904, p. 367), and confirmed by many subsequent observers, that L. donovani gives rise in cultures to a flagellate Herpetomonas-like form. So far as I am aware

no similar observations have been made for L. tropica, and the present memoir is the first account given of the cultural development of Wright's bodies. As will be seen, the result is very similar to that obtained for the Leishman-Donovan body. It is of some interest, however, that the method of cultivation required appears to be quite different in the two cases, a fact which indicates that the transmission and mode of development are different in the two parasites.

E. A. Minchin,
Rovigno, March, 1909.]

(1) Method of cultivation.—Material from the sore was planted in sterile sodium citrate solution (2 per cent.) and in blood-serum (human). Some of the cultures were left at the laboratory temperature (25°–28° C.), and some were incubated at 35° C.

Those incubated at 35° C. and those planted in the sodium citrate solution did not show any growth; on the contrary, after twenty-four, forty-eight, and seventy-two hours they seemed to have disintegrated, so that not even a trace of the original parasites could be seen. In one case (a sodium citrate culture at room-temperature) large staphylococcus-like bodies were seen; they were probably contaminations, and were not investigated further.

On the other hand, in the cultures in blood-serum at laboratory-temperature the parasites went through the development described in detail below; they increase in size, multiply greatly, and finally become Herpetomonas-like flagellates.

(2) The parasites in the sore.—In smears from the juice of the sore stained with Giemsa's stain the parasites are seen in all sorts of shapes—pear-shaped, oval, torpedo-shaped, and even spherical (figs. 1, 2, *a–f*, 3, *a–g*). They are found free outside the corpuscles and also in the large macrophages, in some of which they may occur in considerable numbers (fig. 1). The individual parasites consist of faintly staining protoplasm with macronucleus and micronucleus in various forms. The parasites appear to have a distinct capsule,

shown as a well-defined margin. The length of the body is about one-third that of a red blood-cell; the parasites are therefore a little smaller than those described by James (2), but on the whole the descriptions of Wright (3) and James are applicable to the bodies seen in my specimens.

The micronucleus seems to be more often dot-shaped than rod-shaped, and separation of the dot-shaped micronucleus from the macronucleus can be seen in different stages (figs. 2 and 3). From the study of the smears I am inclined to think that the youngest stage of the parasite is the torpedo-shaped form (fig. 2, *a–c*) with a single nucleus [or with the two nuclei closely apposed], and that the separation of the two nuclei in these spore-like forms comes about after the parasite has entered the macrophage; in other words, that the parasite undergoes development in the macrophage, and that the free parasites showing the typical characters of the two nuclei (fig. 2, *d, e*, fig. 3, *d, f*) are those which have been liberated from a macrophage, either by its disintegration or by ejection from it.

The parasites contained in macrophages are seen to be lodged inside a vacuole or clear space (fig. 1); possibly some sort of secretion is thrown out round the parasite by the macrophage.

The parasites multiply by fission (fig. 3, *e, g*), as described by previous observers. Heart-shaped nuclei are seen in some of the parasites in the smears.

(3) The phases of the development of the parasite in cultures.—After twenty-four hours the cultures, examined in the fresh condition or in stained smears, showed no very obvious increase in the numbers of the parasites, but a sparing distribution in groups of four or eight. The body of the parasite is increased to double its former size, but shows no other difference, except, perhaps, rather better staining of the body-protoplasm.

After forty-eight hours the parasites have grown and multiplied enormously, and are found in masses (fig. 4), visible under the low power. Isolated individuals are also to

be found with high powers, but these are obviously separated from the colonies in the preparation of the smear. The masses consist of great numbers of parasites growing in colonies and entwined with one another. The colonies may be compact and more or less spherical, in which case it is more difficult to make out the shape of the individual parasites; or they may be looser in texture with the individuals more separate (fig. 4).

The parasites have now increased in length to two and a half or three times the diameter of a red blood-corpuscle, and in breadth to about half or two-thirds this diameter. The shape of each individual is like a banana (fig. 4); the contour of the body is well defined, the sides being parallel and the ends rounded; the anterior extremity bearing the micronucleus is more rounded than the other. The parasites are not mobile or amœboid, and for the most part no flagellum can be detected, though in some this organ is already present (fig. 5, *a–c*). The body-protoplasm shows a slightly spongy character, and takes up a deeper stain than in younger forms. The macronucleus is centrally situated and is less compact than in young forms. The micronucleus has shifted to one end of the body and shows a faint semblance of a vacuole round it.

At seventy-two hours the development is complete (fig. 6, *a–j*). The flagellum seems to be formed a few hours after the forty-eight-hour stage as an outgrowth from the micronucleus of a fine filament, which probably shoots out rapidly at the stage when the parasite may be supposed to have reached maturity. Once the flagellum is formed, the individual is liberated and is free to swim away from the colony. Here and there groups of six or more fully developed flagellated individuals are found, entangled by their intertwining flagella.

The body of the parasite is practically the same as described at forty-eight hours, except that it has grown a little longer and stouter, and the distinction between the posterior pointed and the anterior flagellate end is better marked. The flagel-

lum is one and a half times or twice the length of the body of the parasite, and has from six to eight symmetrical undulations. In life the flagellum is very active, and moves very rapidly with a lashing, wave-like, not a corkscrew-like movement; in appearance it strongly resembles a spirochæte.

The parasites are frequently found in pairs (fig. 6, *f–j*) [doubtless indicating fission]. Sometimes a pair consists of a thinner and a thicker individual [compare the splitting off of spirillar forms in L. donovani, described by Leishman and Statham, 'Journ. R.A.M.C.,' iv, 1905, p. 321].

[The foregoing account contains the pith of Dr. Row's first letter to me, and appears to represent the normal healthy development of the parasite in cultures. With the object of obtaining a slower development of the parasite, especially of the stages previous to the formation of the flagellum, material was taken from a case of longer standing, and the parasites were allowed to "stew in their own juice" for three days in a sealed tube at laboratory temperature before cultivating them. The result was a slower development of the parasites in the cultures, with production of peculiar forms probably representing abnormal or degenerative forms of the parasites weakened by the unfavourable conditions of its development. An account of these cultures forms the substance of Dr. Row's second communication to me, and a brief abstract of it follows.—E. A. M.]

The parasite, when derived from an old case (in which the lesion is about to break down into pus and is on the point of ulcerating, and when, consequently, the contents of the lesion are rich in leucocytes and pus), gives rise in cultures to a slow and irregular development, both in numbers and in morphological characters. Under these unfavourable developmental conditions very few typical well-developed and mature flagellates are produced; the products of stunted forms either do not reach the flagellate stage at all, or, if they do so, they are unable to continue their existence long.

The following seems to be the general plan of the development. The parasites begin to elongate into ovoids (fig. 7, *a*)

and then become pear-shaped (fig. 7, *b–h*), with one end pointed. The nucleus divides and division of the body follows (fig. 7, *j–l*). The pear-shaped parasite thus gives rise by fission to a slender form and a stout form (fig. 8, *a*). Each of these divides again twice (fig. 8, *b–i*), so that from one parasite are derived eight flagellates—four long forms and four short and stunted forms (compare fig. 8, *l*). The stunted forms are short-lived, but the long forms persist in cultures of even 120 hours' standing. All the division takes place before the flagellum is formed; after this event there seems to be no further multiplication. [This conclusion can only apply to the stunted cultures; in the more healthy cultures, as stated above, there is evidence of multiplication of the flagellated forms. Moreover, the vast masses of parasites in the forty-eight-hour healthy cultures indicate that here many more than eight flagellates are derived from a single Wright's body.]

Addendum.

[When this memoir was completed and ready to send away I received, on March 27th, a third communication from Dr. Row, containing in concise form his conclusions, which I append here in his own words. Dr. Row exhibited his preparations and read a paper on them at the Medical Congress held in Bombay in February of this year.—E. A. M.]

I conclude that the parasite of the oriental sore (Leishmania tropica) and that of kala-azar (L. donovani), although apparently identical when examined in smears direct from the lesion, are distinct when examined in cultures, and for the following reasons:

(1) The parasite of oriental sore, when fully developed into the flagellate forms under ordinary conditions of culture, is much longer and bigger than that of kala-azar, where one meets with shorter and stouter forms, as a rule.

(2) The parasite of oriental sore is more resistant to external conditions than that of kala-azar; in other words it

is much less delicate, as it is possible to obtain developmental forms up to the flagellate stage from the parasite of oriental sore three days after its removal from the lesion, while the parasite of kala-azar, according to Rogers, dies within twenty-four hours after it leaves the spleen, if not cultured within that period.

(3) The flagellum of the parasite of oriental sore is much longer, and presents more regular wavy undulations than that of kala-azar, where it is shorter with less regular undulations.

(4) Although contamination of the material with extraneous germs is inhibitory to the early developmental progress of the parasite of oriental sore, it is not so destructive to its further development into flagellates as in the case of the parasite of kala-azar, where, according to Rogers, the slightest contamination with staphylococci is sufficient to destroy the culture.

(5) The parasite of oriental sore develops into fully mature flagellate forms between forty-eight and seventy-two hours, while that of kala-azar takes twice as long if not longer.

(6) The best culture medium (according to my results) for the parasite of oriental sore is human blood-serum, by preference that from tuberculous patients; while that for the parasite of kala-azar, according to Rogers, is sodium citrate, 2–10 per cent., in sodium chloride solution 0·8 per cent., mixed with splenic blood.

(7) The optimum temperature for the growth of the parasite of oriental sore is between 25° and 28° C., or even up to 30° C., while for the parasite of kala-azar it is 22° C., or even less, according to Rogers.

[Postscript (July 5th, 1909).—Since the above was sent to press I have been informed by Sir W. B. Leishman that the parasites of oriental sore have also been cultivated by Nicolle, whose memoir, however, I have not been able to see. —E.A.M.]

References.

1. Christophers, S. R.—"Reports on a Parasite Found in Persons Suffering from Enlargement of the Spleen in India," 'Sci. Mem. Officers Govt. India,' Nos. 8, 11, and 15, 1904–5.

2. James, S. R.—"Oriental or Delhi Sore," op. cit., No. 13, 1905.

3. Wright, J. H.—"Protozoa in a Case of Tropical Ulcer (Delhi Sore)" 'Journ. Med. Research, Boston,' x, 1903, p. 472, 4 pls.

EXPLANATION OF PLATE 20,

Illustrating Dr. Row's paper on "The Development of the Parasite of Oriental Sore in Cultures."

[All figures are drawn with the camera lucida to a magnification of 2000 diameters from preparations stained with Giemsa's stain.]

Fig. 1.—Large macrophage containing numerous parasites, from a smear of the juice of the sore.

Fig. 2, *a–f*.—Free parasites from the same smear; *a–c*, young forms with micronucleus and macronucleus closely apposed; *d–f*, parasites probably set free from a macrophage.

Fig. 3, *a–g*.—Free parasites from a smear of an old case, showing various shapes of the body and conditions of the nucleus; *e*, *g*, dividing forms.

Fig. 4.—Mass of parasites from a smear of a culture of forty-eight hours' standing, showing various shapes and sizes of the parasite.

Fig. 5, *a–c*.—Three flagellated individuals from the same preparation as the last figure.

Fig. 6, *a–i*.—Flagellated Herpetomonas-forms from a smear of a culture of seventy-two hours' standing; *a–e*, various forms of single flagellates; *f–i*, parasites in groups and pairs.

Fig. 7, *a–o*.—Parasites from a smear of a retarded culture of twenty-four hours' standing; various stages of the development are seen. Some parasites, such as the two small ones in *g*, have not developed at all; others, such as *k* and *l*, have developed much further and are dividing.

Fig. 8, *a–m*.—Parasites of a smear of a retarded culture of forty-eight hours standing. *a*, division into a stout and a slender form; *b–d*, division of stout forms; *e–i*, division of slender forms; *j*, *k*, groups showing various stages of development; in *j* is seen a parasite not advanced beyond the initial stage; *l*, a group showing a pair of slender forms, recently divided off, and a pair of stout forms in the act of dividing; *m*, two pairs of slender forms (one dividing) with flagella growing out.